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Abstract: OBJECTIVE: The molecular mechanisms underlying the association between obesity (BMI 30 kg/m(2)) and asthma are poorly understood. Since shifts in the fate of bronchial cells due to low-grade systemic inflammation may provide a possible explanation, we investigated whether two of the best documented functional variants in cell cycle control genes modify the obesity-asthma association. METHODS: We genotyped 5930 SAPALDIA cohort participants for the single-nucleotide polymorphisms (SNPs) rs9344 in the cyclin D1 gene (CCND1) and rs1042522 in the gene encoding tumor protein 53 (TP53). We assessed the independent association of these SNPs and obesity with asthma prevalence and incidence. RESULTS: The CCND1 SNP modified the association between obesity and asthma prevalence (p(interaction)= 0.03). The odds ratios (ORs) and 95% confidence intervals (CIs) for reporting a physician diagnosis of asthma at baseline, comparing obese with non-obese participants, were 1.09 (0.51-2.33), 1.64 (0.94-2.88), and 3.51 (1.63-7.53) for GG, GA, and AA genotypes, respectively. We found comparable genotype differences for incident asthma within the 11 years of follow-up. As for the TP53 SNP, the interactions with obesity status with respect to asthma were not statistically significant. CONCLUSIONS: Our results suggest that obesity may contribute to asthma and associated tissue remodeling by modifying the processes related to the CCND1 gene activity.

DOI: https://doi.org/10.3109/02770903.2012.757776

Journal of Asthma, 2013; 50(2): 147–154

ISSN: 0277-0903 print/1532-4303 online
DOI: 10.3109/02770903.2012.757776

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GENETICS

The Association of a Variant in the Cell Cycle Control Gene *CCND1* and Obesity on the Development of Asthma in the Swiss SAPALDIA Study

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Objective. The molecular mechanisms underlying the association between obesity (BMI $\geq$ 30 kg/m^2) and asthma are poorly understood. Since shifts in the fate of bronchial cells due to low-grade systemic inflammation may provide a possible explanation, we investigated whether two of the best documented functional variants in cell cycle control genes modify the obesity–asthma association. *Methods.* We genotyped 5930 SAPALDIA cohort participants for the single-nucleotide polymorphisms (SNPs) rs9344 in the cyclin D1 gene (*CCND1*) and rs1042522 in the gene encoding tumor protein 53 (*TP53*). We assessed the independent association of these SNPs and obesity with asthma prevalence and incidence. *Results.* The *CCND1* SNP modified the association between obesity and asthma prevalence ($p_{interaction} = 0.03$). The odds ratios (ORs) and 95% confidence intervals (CIs) for reporting a physician diagnosis of asthma at baseline, comparing obese with non-obese participants, were 1.09 (0.51–2.33), 1.64 (0.94–2.88), and 3.51 (1.63–7.53) for GG, GA, and AA genotypes, respectively. We found comparable genotype differences for incident asthma within the 11 years of follow-up. As for the *TP53* SNP, the interactions with obesity status with respect to asthma were not statistically significant. *Conclusions.* Our results suggest that obesity may contribute to asthma and associated tissue remodeling by modifying the processes related to the *CCND1* gene activity.

Keywords candidate gene association study, cell proliferation genes, gene–lifestyle interaction, overweight, population-based cohort, single-nucleotide polymorphism

INTRODUCTION

In many parts of the world, obesity prevalence is still on the rise and represents a major public health concern. Obesity increases the risk for later asthma (1), but the underlying etiologic mechanisms for this epidemiologic association remain poorly understood. Explanations based solely on mechanistic effects of excessive body weight seem insufficient, as other aspects of the metabolic syndrome are also associated with impaired lung function (2). Obesity-related inflammatory markers or adipose derived hormones may influence bronchial hyperresponsiveness (BHR) as well as airway inflammation and associated tissue remodeling, as demonstrated in mouse models (3,4). But in human asthma studies, the data in favor of an effect of these circulating molecules on asthma risk remain inconclusive (5,6).

Investigating if gene variants of relevance to inflammatory pathways modify the obesity–asthma association may improve understanding of underlying etiologic mechanisms involved. Currently, there are very few studies available in adults which examine gene–obesity interactions with respect to asthma (7). Candidate genes not yet looked at include those essential to cell cycle control, as airway inflammation and remodeling in asthmatics are associated with altered apoptosis and proliferation of different bronchial cells types such as airway smooth muscle (ASM) and epithelial cells, fibroblasts, as well as cells of the immune system (8–11).

We therefore selected a functionally well-described polymorphism in each of two genes crucial for cell division in order to assess gene–obesity interactions in asthma in a highly focused manner. The cyclin D1 gene (*CCND1)* promotes cell proliferation through cell cycle G1–S phase transition, while the tumor protein 53 gene (*TP53*) is a tumor suppressor gene with a pivotal role in apoptosis. The expression of both genes is commonly altered in numerous cancer types. The single-nucleotide polymorphisms (SNPs) rs9344 [P242P] in *CCND1* and rs1042522 [R72P] in *TP53* are functionally very well characterized and have been associated with various cancer types, including in large meta-analyses (12–14). We previously demonstrated furthermore that these two polymorphisms modified the effect of particulate matter (PM_{10}) air pollution on lung function decline in the SAPALDIA cohort (15). In this same cohort representing the general population from eight Swiss communities, we now investigated whether these two SNPs also affect asthma risk and its relation to obesity. We argue that modification of the asthma–obesity association by variants in cell cycle control genes adds to the evidence for a causal obesity effect, mediated in part by inflammatory pathways.

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METHODS

Study Population

The methods of the SAPALDIA study have been described in detail elsewhere (16,17). In short, the study population consists of a random population sample of white adults aged 18–60 years from eight areas of Switzerland. Totally, 9651 persons participated in the first assessment in 1991, and at the follow-up examination in 2002, 8047 were reassessed with a least a short screening questionnaire. Blood donation and consent to genetic analyses at follow-up was given by 6058 participants of whom 6040 led to successful genotype determination. We could furthermore not include 110 subjects who either had missing information on asthma, smoking status or body mass index (BMI), or reported an asthma diagnosis which had not been confirmed by a physician. Taken together, 3721 individuals of the original study sample could not be considered for the present analysis (hereinafter called non-participants). The analyzed study sample consists hence of a total of 5930 participants. Written consent was obtained from all study participants separately for each assessment procedure. Approval of the study was given by the Swiss Academy of Medical Sciences and the regional ethics committees.

Definition of Smoking Status, Asthma Status, and Obesity

At both surveys, participants underwent a detailed, computer-assisted interview comprising questions about smoking behavior, exposure to environmental tobacco smoke, workplace exposure to dust and fumes, co-morbidities including asthma, medication use, and socio-economic factors. Never smokers were defined as persons who at the time of the interview had smoked less than 20 packs of cigarettes or 360 g of tobacco during their lifetime. Former smokers reported quitting at least one month before the interview. Asthmatics were defined in two ways. First, physician-diagnosed asthma was defined by affirmative answers to "Have you ever had asthma?" and "Was this confirmed by a doctor?" Second, current asthmatics, presenting a subset of those who were diagnosed by a physician, were classified by an affirmative answer to at least one of the following two questions: "Have you had an attack of asthma in the last 12 months?" and "Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?" Incident asthma was defined as a new report of asthma among subjects without asthma at baseline. BMI was divided into four categories: low BMI (BMI < 20 kg/m^2), normal weight (20 kg/m^2 $\leq$ BMI $<$ 25 kg/m^2), overweight (25 kg/m^2 $\leq$ BMI $<$ 30 kg/m^2), and obese (BMI $\geq$ 30 kg/m^2).

Genotyping

DNA extraction from EDTA-buffered whole blood has been previously described (17). Originally, in each of three candidate genes pivotal for cell cycle control (*CCND1*, *TP53,* and *CDKN1A*, the gene encoding cyclin-dependent kinase inhibitor 1A, also called p21), one promising candidate SNP was selected according to its frequency and its reported functionality in cell division and apoptosis (rs9344 in *CCND1*, rs1042522 in *TP53*, and rs1801270 in *CDKN1A*). Genotyping was carried out using fluorescent 5-nuclease real-time PCR methodology (TaqMan, Applera Europe, Rotkreuz, Switzerland) and ABI Prism 7900 sequence detection system (ABI, Rotkreuz, Switzerland). The SNP-specific primers and LNA® dual-labeled fluorogenic probes were designed by Sigma Proligo (Evry, France). A 10% random sample of all DNA samples was re-genotyped, and all genotypes were confirmed. The genotype call rate was >99% for all the three SNPs. Hardy–Weinberg equilibrium was preserved apart from rs1801270 ($p = 0.04$). Since this latter SNP also showed a low minor allele frequency (MAF = 7%), statistical power to investigate effects on asthma and obesity was insufficient, and we excluded this SNP from the analyses of this study.

Statistical Analysis

Main effects of alleles and BMI on physician-diagnosed and current asthma at baseline were assessed using multivariate unconditional logistic regression. *CCND1* and *TP53* genotypes were generally included in a co-dominant fashion using the homozygous genotype of the more frequent allele as the reference category. Due to the limited number of *TP53* CC carriers, this category was combined with the group of heterozygous GC carriers for the analyses of incident asthma, assuming a dominant genetic model. All regression models included study area, sex, age at baseline, and smoking status (never vs. former vs. current) at baseline as covariates. These factors were chosen a priori as they may confound the obesity–asthma association. Models investigating the longitudinal effects of genotypes and change in BMI on incident asthma were additionally adjusted for smoking status at follow-up. Interaction between obesity and genotypes was tested by integrating multiplicative terms in the regression models. Significance levels for two-sided tests of main effects were chosen at $\alpha = 0.05$, and at $\alpha = 0.10$ for tests of effect modification. All statistical analyses were performed using STATA version 10.1 (StataCorp, College Station, TX, USA).

RESULTS

A detailed characterization of the study population can be found in Table 1. Study participants were about half a year older on average and had a slightly lower BMI than non-participants. Totally, 6.2% declared to have ever received an asthma diagnosis by a physician, whereas 2.6% suffered from current asthma. These percentages were higher in non-participants. Never smokers were more likely to be included in this study, confirming that a sample with higher health awareness was attracted to the follow-up examination.

The cross-sectional associations of the genetic polymorphisms in *CCND1* and *TP53* with the prevalence of asthma at baseline are presented in Table 2. The prevalence

TABLE 1.—Baseline characteristics of participants and non-participants in this study.

	Participants, $N = 5930$			Non-participants				
	Mean	SD	%	Mean	SD	%	*N* (with info)	***p*-Value**
Female			50.2			51.8	3721	0.16
Age (years)	41.2	11.4		40.7	12.0		3721	0.06
BMI (kg/m^2)	23.8	3.6		24.2	4.2		3622	**<0.01**
BMI $\geq$ 30 kg/m^2			5.8			9.0	3622	**<0.01**
Physician-diagnosed asthma			6.2			7.8	3624	**<0.01**
Current asthma			2.6			3.4	3616	**0.02**
Smoking-status: never			47.3			38.5	3706	
Smoking-status: former			23.1			21.7	3706	**<0.01**
Smoking-status: current			29.6			39.8	3706	

Notes: Participants include all SAPALDIA follow-up subjects with valid information on sex, age, recruiting area, BMI, smoking status, and who could be successfully genotyped. Answers to questions about self- and physician-diagnosed asthma, as well as recent asthma attacks and asthma medication had to be complete and unambiguous. All SAPALDIA subjects from the original study sample ($N = 9651$) who could not be considered in the present analysis were combined and referred to as non-participants. Equal proportions were tested by chi-square test, equal distributions in age and BMI by Mann–Whitney test. SD, standard deviation; BMI, body mass index.

TABLE 2.—Associations of genetic polymorphisms with asthma prevalence at baseline.

Genotype	*N*	Prevalence (%)	OR (95% CI)	*p*-Value
Physician-diagnosed asthma				
CCND1, rs9344				
GG	1648	7.1	1	
GA	2942	6.1	0.85 (0.66–1.08)	0.17
AA	1340	5.3	0.73 (0.54–0.99)	**0.05**
TP53, rs1042522				
GG	3257	6.0	1	
GC	2291	6.5	1.10 (0.88–1.36)	0.42
CC	382	5.5	0.89 (0.56–1.42)	0.62
Current asthma				
CCND1, rs9344				
GG	1648	2.5	1	
GA	2942	2.6	1.05 (0.71–1.53)	0.82
AA	1340	2.8	1.09 (0.69–1.70)	0.72
TP53, rs1042522				
GG	3257	2.5	1	
GC	2291	3.0	1.21 (0.87–1.68)	0.26
CC	382	2.1	0.82 (0.39–1.70)	0.59

Notes: ORs were based on regression models adjusted for age, sex, study area, and smoking status. OR, odds ratio; CI, confidence interval.

of physician-diagnosed asthma was lower among *CCND1* rs9344 AA genotypes compared to homozygous GG individuals ($p = 0.05$), but this difference could not be detected for current asthma. The polymorphism in *TP53* was not associated with either asthma phenotype.

By dividing the individuals into four BMI categories, we confirmed the well-established association between asthma and obesity (Table 3). Compared to those with BMI between 20 and 25, obese individuals had a 1.81 times higher odds of having an asthma diagnosis by a physician ($p = 0.003$) and a 2.69 times higher odds of suffering from current asthma ($p < .001$). Interestingly, the cross-sectional association between asthma and obesity depended on the genotype of the *CCND1* SNP. The association of obesity with the risk of asthma was restricted to the AA genotype as graphically displayed in Figure 1 for physician-diagnosed asthma. Since low BMI and overweight did not essentially alter the odds of being an asthmatic of normal weight people, we classified all non-obese subjects (BMI < 30 kg/m^2) into one category and compared them with obese subjects. Overall, obese subjects were 1.78 times (95% confidence interval (CI) 1.22–2.61) more likely to report an asthma diagnosis confirmed by a physician. For AA carriers of rs9344 in *CCND1*, this odds ratio (OR) increased to 3.51 (95% CI 1.63–7.53). The equivalent ORs for current asthma were even more pronounced (overall OR 2.65, 95% CI 1.60–4.40; and for AA carriers OR 7.17, 95% CI 2.89–17.83). Interaction terms between *CCND1* genotype and obesity were significant for both asthma definitions ($p = 0.03$ for physician-diagnosed asthma and $p = 0.1$ for current asthma). As for rs1042522 in *TP53*, obesity tended to be more strongly associated with the risk of asthma in homozygotes for the G allele than in heterozygotes, but these differences were based on very small sample sizes. When including BMI as a continuous variable, we observed similar patterns of associations.

Table 4 presents the longitudinal association of asthma incidence with the change in obesity status during follow-up. Obesity development was categorized into three distinct groups: participants who were never obese, participants who reached obesity at follow-up, and participants who were obese at both times of data collection. The latter group had the highest risk for developing asthma between baseline and follow-up (OR 2.05 for physician-diagnosed asthma and OR 2.58 for current asthma, compared to those who were never obese). Stratification by *CCND1* genotypes modified this risk. We observed the highest risk among AA carriers ($p < 0.001$ for both asthma definitions). Despite the wide confidence intervals, the *p*-values for interaction between *CCND1* genotype and obesity development in relation to asthma incidence were marginally statistically significant ($p = 0.09$ for physician-diagnosed asthma and $p = 0.03$ for current asthma). The *TP53* SNP did not modify the association between obesity development and asthma incidence.

Additional adjustment for atopy (positive skin prick test), smoking intensity, early childhood infection, family history of asthma, and exposure to gas, dust, or fumes did not substantially alter the results (data not shown).

TABLE 3.—Cross-sectional association of BMI with asthma risk at baseline, overall and stratified by *CCND1* and *TP53* genotypes.

		Physician-diagnosed asthma				**Current asthma**			
	BMI	Yes/no	OR	95% CI	*p*-Value	Yes/no	OR	95% CI	*p*-Value
All	<20	40/712	0.85	0.59–1.22	0.38	16/736	0.80	0.45 to 1.39	0.42
	20–25	195/3053	1	ref		81/3167	1	ref	
	25–30	98/1490	1.09	0.84–1.41	0.53	40/1548	1.11	0.75 to 1.66	0.60
	≥30	33/309	1.81	1.22–2.68	**0.003**	19/323	2.69	1.59 to 4.56	**<0.001**
	<30	333/5255	1	ref		137/5451	1	ref	
	≥30	33/309	1.78	1.22–2.61	**0.003**	19/323	2.65	1.60 to 4.40	**<0.001**
	Continuous	366/5564	1.05	1.02–1.08	**0.003**	156/5774	1.08	1.04 to 1.12	**<0.001**
CCND1, rs9344 GG	<30	109/1433	1	ref		39/1503	1	ref	
	≥30	8/98	1.09	0.51–2.33	0.83	3/103	1.20	0.35 to 4.09	0.77
	Continuous	117/1531	0.98	0.93–1.04	0.55	42/1606	0.99	0.90 to 1.09	0.83
GA	<30	163/2607	1	ref		69/2701	1	ref	
	≥30	15/157	1.64	0.94–2.88	0.08	8/164	2.07	0.97 to 4.44	0.06
	Continuous	178/2764	1.06	1.02–1.10	**0.005**	77/2865	1.08	1.02 to 1.14	**0.006**
AA	<30	61/1215	1	ref		29/1247	1	ref	
	≥30	10/54	3.51	1.63–7.53	**0.001**	8/56	7.17	2.89 to 17.83	**<0.001**
	Continuous	71/1269	1.11	1.04–1.19	**0.003**	37/1303	1.17	1.07 to 1.28	**<0.001**
TP53, rs1042522 GG	<30	172/2887	1	ref		68/2991	1	ref	
	≥30	23/175	2.41	1.50–3.87	**<0.001**	12/186	3.27	1.69 to 6.30	**<0.001**
	Continuous	195/3062	1.06	1.02–1.11	**0.002**	80/3177	1.08	1.02 to 1.14	**0.01**
GC	<30	142/2027	1	ref		63/2106	1	ref	
	≥30	8/114	1.04	0.49–2.19	0.92	5/117	1.59	0.62 to 4.11	0.34
	Continuous	150/2141	1.01	0.96–1.06	0.68	68/2223	1.06	0.99 to 1.13	0.08
CC	<30	19/341	1	ref		6/354	1	ref	
	≥30	2/20	2.45	0.46–13.03	0.29	2/20	8.29	1.05 to 65.36	**0.05**
	Continuous	21/361	1.16	1.01–1.32	**0.03**	8/374	1.34	1.06 to 1.69	**0.02**

Notes: Regression models were adjusted for age, sex, study area and smoking status. *p*-Values for interaction between *CCND1* genotype and obesity were **0.03** (physician-diagnosed asthma) and **0.01** (current asthma). *p*-Values for interaction between *CCND1* genotype and continuous BMI were **0.02** (physician-diagnosed asthma) and **0.03** (current asthma). *p*-Values for interaction between *TP53* genotype and obesity were 0.14 (physician-diagnosed asthma) and 0.64 (current asthma). *p*-Values for interaction between *TP53* genotype and continuous BMI were 0.86 (physician-diagnosed asthma) and 0.50 (current asthma). BMI, body mass index; OR, odds ratio; CI, confidence interval.

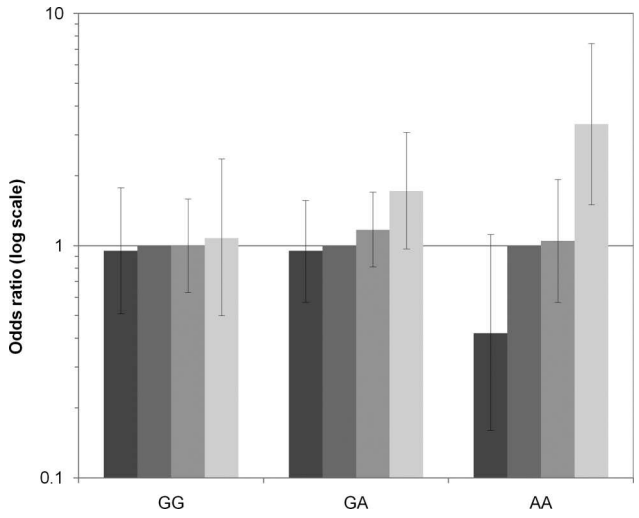

FIGURE 1.—Odds ratios (bars) and 95% confidence intervals (lines) for the cross-sectional association of body mass index (BMI) category and physician-diagnosed asthma stratified by *CCND1* genotype. BMI categories represent from darker to lighter color: <20 kg/m^2, 20–25 kg/m^2, 25–30 kg/m^2, and ≥30 kg/m^2. The reference group corresponded to individuals with normal weight (BMI 20–25 kg/m^2).

DISCUSSION

We show for the first time that the cross-sectional and longitudinal association of obesity and asthma is modified by a SNP in a gene which exhibits a central role in cell cycle control. The associations appear to be stronger among subjects homozygous for the *CCND1* A allele in rs9344, the same genotype which in numerous studies including a large meta-analysis predisposed to various cancer subtypes across different ethnic populations (13). According to our study, these participants' lung tissue may be more prone to remodeling in response to obesity-related processes.

Asthma in Obese People

Our results confirm the well-established association of obesity with asthma risk. Current knowledge include that the obesity–asthma association decreases with progressive age (18) and may in part be mediated by low-grade systemic inflammation (19). Twin studies suggest that shared genetic pathways for asthma and obesity may partly explain the observed associations (20). Candidate gene studies and, more recently, genome-wide association studies have identified a large number of genes associated with either of the two conditions, but these genes do not seem to have a substantial impact on both phenotypes simultaneously (21). Candidate genes which seemed to be important for the asthma risk in obese people or for the higher BMI in asthmatics encompass *LEP*, *TNFA*, and *PRKCA*, regions encoding the adipose tissue derived

TABLE 4.—Longitudinal association of change in obesity status during follow-up with asthma incidence, overall and stratified by *CCND1* and *TP53* genotypes.

		Incidence of physician-diagnosed asthma ($N = 174$ cases)				Incidence of current asthma ($N = 115$ cases)			
BMI (baseline/follow up)		Yes/no	OR	95% CI	*p*-Value	Yes/no	OR	95% CI	*p*-Value
All	<30/<30	145/4481	1	ref		89/4735	1	ref	
	<30/≥30	15/551	0.91	0.53–1.57	0.73	15/588	1.47	0.84–2.57	0.18
	≥30/≥30	14/266	2.05	1.15–3.66	**0.02**	11/282	2.58	1.34–4.96	**0.005**
CCND1, rs9344									
GG	<30/<30	42/1231	1	ref		29/1309	1	ref	
	<30/≥30	2/140	0.46	0.11–1.94	0.29	1/158	0.30	0.04–2.28	0.24
	≥30/≥30	4/85	1.90	0.63–5.74	0.25	3/90	1.73	0.50–6.05	0.41
GA	<30/<30	80/2212	1	ref		45/2344	1	ref	
	<30/≥30	11/272	1.25	0.65–2.40	0.51	10/287	2.01	0.99–4.09	0.05
	≥30/≥30	5/138	1.18	0.46–3.00	0.73	4/146	1.72	0.60–4.94	0.32
AA	<30/<30	23/1038	1	ref		15/1082	1	ref	
	<30/≥30	2/139	0.68	0.16–3.01	0.61	4/143	2.24	0.71–7.11	0.17
	≥30/≥30	5/43	8.86	2.82–27.81	**<0.001**	4/46	11.85	3.32–42.24	**<0.001**
TP53, rs1042522									
GG	<30/<30	85/2443	1	ref		53/2576	1	ref	
	<30/≥30	10/318	0.94	0.48–1.85	0.73	9/341	1.27	0.62–2.63	0.62
	≥30/≥30	9/153	2.10	1.01–4.37	**0.02**	8/164	2.85	1.29–6.26	**0.004**
GC/CC	<30/<30	60/2038	1	ref		36/2159	1	ref	
	<30/≥30	5/233	0.81	0.32–2.07	0.66	6/247	1.68	0.69–4.12	0.25
	≥30/≥30	5/113	1.90	0.73–4.92	0.19	3/118	1.96	0.58–6.64	0.28

Notes: Included are participants without asthma at baseline ($N = 5472$ for physician-diagnosed asthma and $N = 5720$ for current asthma). Regression models were adjusted for age, sex, study area, and smoking status at baseline and follow-up. GC and CC genotypes of rs1042522 were taken together since the number of these genotypes was very low. *p*-Values for interaction between *CCND1* genotype and obesity development were **0.09** (incidence of physician-diagnosed asthma) and **0.03** (incidence of current asthma). *p*-Values for interaction between *TP53* genotype and obesity development were 0.79 (incidence of physician-diagnosed asthma) and 0.68 (incidence of current asthma). BMI, body mass index; OR, odds ratio; CI, confidence interval.

molecules leptin and tumor necrosis factor alpha (TNFα) as well as the ubiquitously expressed protein kinase C alpha, a factor associated with adipocyte differentiation and insulin signaling (7,22,23). Cell cycle control genes have so far not been investigated in this respect, but their importance in inflammatory processes confer them etiologic plausibility to potentially modify the obesity–asthma relationship. Interestingly, an in vitro study with asthma serum-sensitized human ASM cells recently found protein kinase C alpha to upregulate cyclin D1 expression (24).

Asthma: High Inflammatory Stress and Altered Cell Cycle Control

In the present study, the *CCND1* polymorphism rs9344 was associated with physician-diagnosed asthma at borderline significance level, but not with current asthma. This effect seems hence to originate from a history of asthma or a form of asthma which does not manifest itself in attacks on a regular basis. The inflammatory processes in the bronchial tissue of untreated asthma lead to structural changes in the airways. Gene array techniques comparing gene expression profiles of various cell types in bronchial tissue as well as in peripheral blood between asthmatics and healthy controls exhibit significant differences in numerous genes (25). For instance, atopic asthma has been associated with both reduced apoptosis of airway inflammatory cells as well as reduced net *TP53* activity and thus reduced apoptosis of peripheral blood mononuclear cells (8,26). ASM mass and cell proliferation is increased in asthma (9), and this feature seems to involve *CCND1* the expression of which was elevated in asthma serum-sensitized human ASM cells (24). A significant suppression of bronchial epithelial cell proliferation associated with increased *CDKN1A* expression was observed in the asthmatic bronchial epithelium in humans (27). Inherited differences in the cell cycle response to inflammatory and other oxidative stressors could hence in part underlie asthma etiology. In agreement, asthma risk factors with oxidative properties like tobacco smoke or air pollutants were shown to alter cell proliferation in the airways (28) and to alter lung function in a manner dependent on cell cycle gene variants (15).

Obesity and Molecular Pathways of Potential Relevance to Asthma

Obesity shares with some of the inhaled asthma triggers the capacity to induce a status of low-grade systemic inflammation. Diseases associated with over-nutrition are characterized by alterations in circulating levels of inflammatory cytokines and adipose derived hormones (29). This so-called metabolic inflammation interferes with the regulation of intracellular molecular pathways that include cell cycle control mechanisms and could therefore also play a role in asthma etiology.

The PI3K/Akt signal pathway is activated by many of the factors which are altered in obesity and lead to increased cell survival (30). This pathway is also crucial in asthma pathophysiology. Namely, it has recently been

shown that osteopontin, an extracellular matrix protein upregulated in the lungs of asthmatics, activates the PI3K/Akt pathway, and is associated with airway remodeling and disease severity in human asthma (31,32).

The transcription factor nuclear factor kappa B (NF-κB), one of the downstream targets of PI3K/Akt, interacts with several adipose-derived molecules. For instance, leptin stimulates and adiponectin reduces NF-κB signaling in endothelial cells (33,34), while the cytokine TNFα has been shown to interact with NF-κB signaling in ASM cells (35). We previously reported a complex joint effect of obesity with a *TNFA* polymorphism on asthma in two large cohort studies including SAPALDIA (7). NF-κB also exhibits a central role in the proliferating airway epithelium of asthmatics (36). Its interplay with different cell cycle control genes including *TP53* and *CCND1* is diverse (37,38). Compatible with this picture, leptin and adiponectin have also been found to be able to modulate expression levels of cyclin D1 or p53 in different types of cancer cells (39,40).

Both adiponectin and leptin are stimuli of the AMP-activated protein kinase (AMPK) (41). AMPK serves as an energy sensor and suppresses cell proliferation in non-malignant and tumor cells by interacting with the cell cycle machinery (42). As cell growth and proliferation are energy-intensive processes, AMPK may act as an energy checkpoint, permitting progression through the cell cycle only in the presence of sufficient energy reserves. Interestingly, metformin, an AMPK activator used in the treatment of obesity-related diabetes, was found to inhibit ASM cell proliferation (43).

Since obesity is also an established risk factor for colon cancer and many of the described pathways and mechanisms are also discussed in the etiology of that disease (30), results from colon cancer epidemiology are relevant to the interpretation of our findings, as they support the notion that cell cycle genes interact with inflammatory and other oxidative stressors including obesity. We reported an interacting effect on the risk of colorectal cancer for the *CCND1* SNP studied in this work with dietary antioxidants and proteins exhibiting antioxidative properties (44). Taken together, these findings corroborate the view that (a) obesity-mediated inflammation may directly affect cell cycle control, proliferation, and apoptosis in a variety of different tissues and that (b) the efficiency of these effects can depend on polymorphisms in cell cycle control master regulatory genes.

Strengths and Limitations of This Study

This study has several advantages. The study sample was selected to be representative of the adult population in the eight study areas. The study population is well characterized and comparatively large. Population stratification is at best of minor influence since participants had to be local residents for at least 3 years prior to the first survey, had to show a good command of one of the national languages, and there was no difference in genotype frequencies between Swiss and non-Swiss citizens ($p = 0.88$ for rs9344 in *CCND1* and $p = 0.26$ for rs1042522 in *TP53*). Moreover, stratification of the associations by study center or language region did not materially alter the main findings. The assessment of asthma relied on internationally validated questions identical to those used in the European Respiratory Health Survey (45).

Nevertheless, some limitations apply. Like in many studies on asthma the definitions of asthma relied solely on the self-report of asthma diagnosis, attacks, and medications. Asthma status may therefore be subject to misclassification. Furthermore, there is indication that obesity-related asthma is a clearly distinct phenotype from general asthma and should be addressed differently. In fact, traditional inflammatory mechanisms in the airways have not been found to be relevant (6) and common asthma medication is far less helpful in obese subjects (46). Moreover, the usually observed asthma remission after weight loss (47) does not seem to support extensive airway remodeling. However, it has been recently demonstrated that airway remodeling may persist in asthmatics with complete asthma remission (48).

We cannot exclude participation bias. Smokers and patients with asthma and related phenotypes were less likely to participate at follow-up. Respiratory health of study participants is therefore slightly better than in non-participants. However, unless participation of asthmatics and non-asthmatics at baseline and follow-up was influenced by one of the genotypes investigated, substantial bias of the results due to non-participation is unlikely. A further limitation is the absence of adequate data on physical activity. This does not allow us to properly differentiate between modification of obesity and physical activity by the genetic polymorphisms investigated.

The study focused on selected SNPs in the genes of interest by giving priority to SNPs intensively studied in cancer. The *CCND1* rs9344 A allele leads to modulated splicing and consequently elevated production of the cyclin D1b isoform, a transcript with a higher cellular transformation potential (49,50). Even though this isoform is not solely dependent on rs9344, this polymorphism has been associated with different types of cancer (12). Apart from the high-penetrance mutations in *TP53* that lead to Li–Fraumeni syndrome, the non-synonymous SNP rs1042522 is arguably the most likely with functional relevance (14). The G allele is more powerful in inducing apoptosis (51), and an analysis summarizing several studies looking at different types of cancer could find a statistically marginally higher risk for carriers of the C allele (52). Further functional studies on the protein level are needed to clarify how the *CCND1* and *TP53* polymorphisms influence the cell division activity.

Finally, despite the large sample size of our study, the statistical power became limited in some of the stratified analyses, especially for the *TP53* genotypes and asthma incidence. Replication of the results in an independent cohort study is therefore essential, and with the help of pathway analysis involving many more SNPs and genes, the importance of cell cycle control activity in the asthma–obesity association could be more rigorously assessed.

Conclusion

Since obesity is a worldwide rapidly growing phenomenon, it is of high public health relevance to clarify causality and mechanisms of its association with asthma. Our results, if confirmed, suggest that obesity may contribute to asthma and associated tissue remodeling by modifying processes related to the *CCND1* gene activity.

Acknowledgments

The authors thank the following: The SAPALDIA team; study directorate: T Rochat (p), JM Gaspoz (c), N Künzli (e/exp), LJS Liu (exp), NM Probst-Hensch (e/g), C Schindler (s). Scientific team: JC Barthélémy (c), W Berger (g), R Bettschart (p), A Bircher (a), G Bolognini (p), O Brändli (p), C Brombach (n), M Brutsche (p), L Burdet (p), M Frey (p), U Frey (pd), MW Gerbase (p), D Gold (e/c/p), E de Groot (c), W Karrer (p), R Keller (p), B Knöpfli (p), B Martin (pa), D Miedinger (o), U Neu (exp), L Nicod (p), M Pons (p), F Roche (c), T Rothe (p), E Russi (p), P Schmid-Grendelmeyer (a), A Schmidt-Trucksäss (pa), A Turk (p), J Schwartz (e), D. Stolz (p), P Straehl (exp), JM Tschopp (p), A von Eckardstein (cc), E Zemp Stutz (e). Scientific team at coordinating centers: M Adam (e/g), E Boes (g), PO Bridevaux (p), D Carballo (c), E Corradi (e), I Curjuric (e), J Dratva (e), A Di Pasquale (s), L Grize (s), D Keidel (s), S Kriemler (pa), A Kumar (g), M Imboden (g), N Maire (s), A Mehta (e), F Meier (e), H Phuleria (exp), E Schaffner (s), GA Thun (g) A Ineichen (exp), M Ragettli (exp), M Ritter (exp), T Schikowski (e), G Stern (pd), M Tarantino (s), M Tsai (exp), M Wanner (pa). (a) Allergology, (c) cardiology, (cc) clinical chemistry, (e) epidemiology, (exp) exposure, (g) genetic and molecular biology, (m) meteorology, (n) nutrition, (o) occupational health, (p) pneumology, (pa) physical activity, (pd) pediatrics, (s) statistics. Administrative staff: C Gabriel, R Gutknecht. The study could not have been done without the help of the study participants, technical and administrative support and the medical teams and field workers at the local study sites. Local fieldworkers: Aarau: S Brun, G Giger, M Sperisen, M Stahel. Basel: C Bürli, C Dahler, N Oertli, I Harreh, F Karrer, G Novicic, N Wyttenbacher. Davos: A Saner, P Senn, R Winzeler, Geneva: F Bonfils, B Blicharz, C Landolt, J Rochat. Lugano: S Boccia, E Gehrig, MT Mandia, G Solari, B Viscardi. Montana: AP Bieri, C Darioly, M Maire. Payerne: F Ding, P Danieli A Vonnez. Wald: D Bodmer, E Hochstrasser, R Kunz, C Meier, J Rakic, U Schafroth, A Walder.

Declaration of Interest

The authors declare that they have no competing interests.

SAPALDIA is supported by the Swiss National Science Foundation (grants no, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247–065896, 3100–059302, 3200–052720, 3200–042532, 4026–028099, 3233–054996, PDFMP3-123171), the Federal Office for Forest, Environment and Landscape, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, Zurich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais and Zurich, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA.

References

1. Beuther DA, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. Am J Respir Crit Care Med 2007; 175:661–666.
2. Leone N, Courbon D, Thomas F, Bean K, Jego B, Leynaert B, Guize L, Zureik M. Lung function impairment and metabolic syndrome: the critical role of abdominal obesity. Am J Respir Crit Care Med 2009; 179:509–516.
3. Shore SA, Schwartzman IN, Mellema MS, Flynt L, Imrich A, Johnston RA. Effect of leptin on allergic airway responses in mice. J Allergy Clin Immunol 2005; 115:103–109.
4. Medoff BD, Okamoto Y, Leyton P, Weng M, Sandall BP, Raher MJ, Kihara S, Bloch KD, Libby P, Luster AD. Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. Am J Respir Cell Mol Biol 2009; 41:397–406.
5. Sood A, Ford ES, Camargo CAJr. Association between leptin and asthma in adults. Thorax 2006; 61:300–305.
6. Jartti T, Saarikoski L, Jartti L, Lisinen I, Jula A, Huupponen R, Viikari J, Raitakari OT. Obesity, adipokines and asthma. Allergy 2009; 64:770–777.
7. Castro-Giner F, Kogevinas M, Imboden M, de Cid R, Jarvis D, Machler M, Berger W, Burney P, Franklin KA, Gonzalez JR, Heinrich J, Janson C, Omenaas E, Pin I, Rochat T, Sunyer J, Wjst M, Anto JM, Estivill X, Probst-Hensch NM. Joint effect of obesity and TNFA variability on asthma: two international cohort studies. Eur Respir J 2009; 33:1003–1009.
8. Vignola AM, Chanez P, Chiappara G, Siena L, Merendino A, Reina C, Gagliardo R, Profita M, Bousquet J, Bonsignore G. Evaluation of apoptosis of eosinophils, macrophages, and T lymphocytes in mucosal biopsy specimens of patients with asthma and chronic bronchitis. J Allergy Clin Immunol 1999; 103:563–573.
9. Johnson PR, Roth M, Tamm M, Hughes M, Ge Q, King G, Burgess JK, Black JL. Airway smooth muscle cell proliferation is increased in asthma. Am J Respir Crit Care Med 2001; 164:474–477.
10. Kraft M, Lewis C, Pham D, Chu HW. IL-4, IL-13, and dexamethasone augment fibroblast proliferation in asthma. J Allergy Clin Immunol 2001; 107:602–606.
11. Cohen L, Xueping E, Tarsi J, Ramkumar T, Horiuchi TK, Cochran R, DeMartino S, Schechtman KB, Hussain I, Holtzman MJ, Castro M. Epithelial cell proliferation contributes to airway remodeling in severe asthma. Am J Respir Crit Care Med 2007; 176:138–145.
12. Knudsen KE, Diehl JA, Haiman CA, Knudsen ES. Cyclin D1: polymorphism, aberrant splicing and cancer risk. Oncogene 2006; 25:1620–1628.
13. Pabalan N, Bapat B, Sung L, Jarjanazi H, Francisco-Pabalan O, Ozcelik H. Cyclin D1 Pro241Pro (CCND1-G870A) polymorphism is associated with increased cancer risk in human populations: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2008; 17:2773–2781.
14. Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer 2009; 9:95–107.
15. Imboden M, Schwartz J, Schindler C, Curjuric I, Berger W, Liu SL, Russi EW, Ackermann-Liebrich U, Rochat T, Probst-Hensch NM. Decreased PM10 exposure attenuates age-related lung function decline: Genetic variants in p53, p21, and CCND1 modify this effect. Environ Health Perspect 2009; 117:1420–1427.
16. Martin BW, Ackermann-Liebrich U, Leuenberger P, Kunzli N, Stutz EZ, Keller R, Zellweger JP, Wuthrich B, Monn C, Blaser K, Bolognini G, Bongard JP, Brandli O, Braun P, Defila C, Domenighetti G, Grize L,

Karrer W, Keller-Wossidlo H, Medici TC, Peeters A, Perruchoud AP, Schindler C, Schoeni MH, Schwartz J, Solari G, Tschopp JM, Villiger B. SAPALDIA: Methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. Soz Praventivmed 1997; 42:67–84.
17. Ackermann-Liebrich U, Kuna-Dibbert B, Probst-Hensch NM, Schindler C, Felber Dietrich D, Stutz EZ, Bayer-Oglesby L, Baum F, Brandli O, Brutsche M, Downs SH, Keidel D, Gerbase MW, Imboden M, Keller R, Knopfli B, Kunzli N, Nicod L, Pons M, Staedele P, Tschopp JM, Zellweger JP, Leuenberger P. Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991–2003: methods and characterization of participants. Soz Praventivmed 2005; 50:245–263.
18. Lang JE, Hossain J, Dixon AE, Shade D, Wise RA, Peters SP, Lima JJ. Does age impact the obese asthma phenotype? Longitudinal asthma control, airway function, and airflow perception among mild persistent asthmatics. Chest 2011; 140:1524–1533.
19. Shore SA. Obesity, airway hyperresponsiveness, and inflammation. J Appl Physiol 2010; 108:735–743.
20. Hallstrand TS, Fischer ME, Wurfel MM, Afari N, Buchwald D, Goldberg J. Genetic pleiotropy between asthma and obesity in a community-based sample of twins. J Allergy Clin Immunol 2005; 116:1235–1241.
21. Melen E, Himes BE, Brehm JM, Boutaoui N, Klanderman BJ, Sylvia JS, Lasky-Su J. Analyses of shared genetic factors between asthma and obesity in children. J Allergy Clin Immunol 2010; 126(631–637): e631-638.
22. Szczepankiewicz A, Breborowicz A, Sobkowiak P, Popiel A. Are genes associated with energy metabolism important in asthma and BMI?. J Asthma 2009; 46:53–58.
23. Murphy A, Tantisira KG, Soto-Quiros ME, Avila L, Klanderman BJ, Lake S, Weiss ST, Celedon JC. PRKCA: A positional candidate gene for body mass index and asthma. Am J Hum Genet 2009; 85:87–96.
24. Du CL, Xu YJ, Liu XS, Xie JG, Xie M, Zhang ZX, Zhang J, Qiao LF. Up-regulation of cyclin D1 expression in asthma serum-sensitized human airway smooth muscle promotes proliferation via protein kinase C alpha. Exp Lung Res 2010; 36:201–210.
25. Hansel NN, Diette GB. Gene expression profiling in human asthma. Proc Am Thorac Soc 2007; 4:32–36.
26. Brutsche MH, Brutsche IC, Wood P, Brass A, Morrison N, Rattay M, Mogulkoc N, Simler N, Craven M, Custovic A, Egan JJ, Woodcock A. Apoptosis signals in atopy and asthma measured with cDNA arrays. Clin Exp Immunol 2001; 123:181–187.
27. Puddicombe SM, Torres-Lozano C, Richter A, Bucchieri F, Lordan JL, Howarth PH, Vrugt B, Albers R, Djukanovic R, Holgate ST, Wilson SJ, Davies DE. Increased expression of p21(waf) cyclin-dependent kinase inhibitor in asthmatic bronchial epithelium. Am J Respir Cell Mol Biol 2003; 28:61–68.
28. Broekema M, ten Hacken NH, Volbeda F, Lodewijk ME, Hylkema MN, Postma DS, Timens W. Airway epithelial changes in smokers but not in ex-smokers with asthma. Am J Respir Crit Care Med 2009; 180:1170–1178.
29. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005; 115:911–919; quiz 920.
30. Huang XF, Chen JZ. Obesity, the PI3K/Akt signal pathway and colon cancer. Obes Rev 2009; 10:610–616.
31. Simoes DC, Xanthou G, Petrochilou K, Panoutsakopoulou V, Roussos C, Gratziou C. Osteopontin deficiency protects against airway remodeling and hyperresponsiveness in chronic asthma. Am J Respir Crit Care Med 2009; 179:894–902.
32. Samitas K, Zervas E, Vittorakis S, Semitekolou M, Alissafi T, Bossios A, Gogos H, Economidou E, Lotvall J, Xanthou G, Panoutsakopoulou V, Gaga M. Osteopontin expression and relation to disease severity in human asthma. Eur Respir J 2011; 37:331–341.
33. Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. FASEB J 1999; 13:1231–1238.
34. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000; 102:1296–1301.
35. Amrani Y, Chen H, Panettieri RAJr. Activation of tumor necrosis factor receptor 1 in airway smooth muscle: a potential pathway that modulates bronchial hyper-responsiveness in asthma?. Respir Res 2000; 1:49–53.
36. Janssen-Heininger YM, Poynter ME, Aesif SW, Pantano C, Ather JL, Reynaert NL, Ckless K, Anathy V, van der Velden J, Irvin CG, van der Vliet A. Nuclear factor kappaB, airway epithelium, and asthma: avenues for redox control. Proc Am Thorac Soc 2009; 6:249–255.
37. Webster GA, Perkins ND. Transcriptional cross talk between NF-kappaB and p53. Mol Cell Biol 1999; 19:3485–3495.
38. Witzel II, Koh LF, Perkins ND. Regulation of cyclin D1 gene expression. Biochem Soc Trans 2010; 38:217–222.
39. Chen C, Chang YC, Liu CL, Chang KJ, Guo IC. Leptin-induced growth of human ZR-75-1 breast cancer cells is associated with up-regulation of cyclin D1 and c-Myc and down-regulation of tumor suppressor p53 and p21WAF1/CIP1. Breast Cancer Res Treat 2006; 98:121–132.
40. Mistry T, Digby JE, Desai KM, Randeva HS. Leptin and adiponectin interact in the regulation of prostate cancer cell growth via modulation of p53 and bcl-2 expression. BJU Int 2008; 101:1317–1322.
41. Luo Z, Saha AK, Xiang X, Ruderman NB. AMPK the metabolic syndrome and cancer. Trends Pharmacol Sci 2005; 26:69–76.
42. Motoshima H, Goldstein BJ, Igata M, Araki E. AMPK and cell proliferation – AMPK as a therapeutic target for atherosclerosis and cancer. J Physiol 2006; 574:63–71.
43. Ratnovsky A, Mellema M, An SS, Fredberg JJ, Shore SA. Airway smooth muscle proliferation and mechanics: Effects of AMP kinase agonists. Mol Cell Biomech 2007; 4:143–157.
44. Probst-Hensch NM, Sun CL, Van Den Berg D, Ceschi M, Koh WP, Yu MC. The effect of the cyclin D1 (CCND1) A870G polymorphism on colorectal cancer risk is modified by glutathione-S-transferase polymorphisms and isothiocyanate intake in the Singapore Chinese Health Study. Carcinogenesis 2006; 27:2475–2482.
45. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J 1994; 7:954–960.
46. Sutherland ER, Goleva E, Strand M, Beuther DA, Leung DY. Body mass and glucocorticoid response in asthma. Am J Respir Crit Care Med 2008; 178:682–687.
47. Eneli IU, Skybo T, Camargo CAJr. Weight loss and asthma: a systematic review. Thorax 2008; 63:671–676.
48. Broekema M, Timens W, Vonk JM, Volbeda F, Lodewijk ME, Hylkema MN, Ten Hacken NH, Postma DS. Persisting remodeling and less airway wall eosinophil activation in complete remission of asthma. Am J Respir Crit Care Med 2011; 183:310–316.
49. Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. Oncogene 1995; 11:1005–1011.
50. Solomon DA, Wang Y, Fox SR, Lambeck TC, Giesting S, Lan Z, Senderowicz AM, Conti CJ, Knudsen ES. Cyclin D1 splice variants. Differential effects on localization, RB phosphorylation, and cellular transformation. J Biol Chem 2003; 278:30339–30347.
51. Dumont P, Leu JI, Della Pietra AC3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003; 33:357–365.
52. van Heemst D, Mooijaart SP, Beekman M, Schreuder J, de Craen AJ, Brandt BW, Slagboom PE, Westendorp RG. Variation in the human TP53 gene affects old age survival and cancer mortality. Exp Gerontol 2005; 40:11–15.